REVIEW ARTICLE

Cancer cell metastasis; perspectives from the focal adhesions

Lefteris C Zacharia[1], Vasiliki Gkretsi[2]*

[1]Department of Life and Health Sciences, University of Nicosia, Cyprus

[2]Department of Biomedical Research and Technology, Institute for Research and Technology-Thessaly (I.RE.TE.TH), Centre for Research and Technology-Hellas (CE.R.T.H), Greece

Abstract: In almost all cancers, most patients die from metastatic disease and not from the actual primary tumor. That is why addressing the problem of metastasis is of utmost importance for the successful treatment and improved survival of cancer patients. Metastasis is a complex process that ultimately leads to cancer cells spreading from the tumor to distant sites of the body. During this process, cancer cells tend to lose contact with the extracellular matrix (ECM) and neighboring cells within the primary tumor, and are thus able to invade surrounding tissues. Hence, ECM and the ECM-associated adhesion proteins play a critical role in the metastatic process. This review will focus on recent literature regarding interesting and novel molecules at the cell-ECM adhesion sites, namely migfilin, mitogen-inducible gene-2 (Mig-2) and Ras suppressor-1 (RSU-1), that are also critically involved in cancer cell metastasis, emphasizing on data from experiments performed *in vitro* in breast cancer and hepatocellular carcinoma cell lines as well as human breast cancer tissue samples.

Keywords: apoptosis; breast cancer; cell-matrix adhesions; Fascin-1; hepatocellular carcinoma; invasion; metastasis; migfilin; PINCH-1; PUMA; Ras suppressor-1; VASP

In most cancer types, including breast cancer (BC) and hepatocellular carcinoma (HCC), a large percentage of patients die from metastatic disease[1,2] and not from complications related to the original primary tumor. That is why addressing the problem of metastasis is of utmost importance for the successful treatment and improved survival of cancer patients. Metastasis is a complex process that ultimately leads to cancer cell spreading through tissues in the whole body. During the metastatic process, as cancer cells accumulate mutations or other molecular signals, they become more malignant and tend to easily lose contact with the extracellular matrix (ECM) and neighboring cells within the primary tumor. Hence, they start to invade surrounding tissues. Both cell-cell adhesion and cell-ECM adhesions get deregulated promoting cancer cell aggressiveness[3]. In fact, communication between cells and the ECM, and between neighboring cells is severely disrupted in more aggressive and invasive cells[4-6]. Hence, ECM, integrins and the ECM-associated adhesion proteins play a critical role in this process[4].

This review will focus on interesting and novel molecules at the cell-ECM adhesion sites that are also critically involved in cancer cell metastasis, based on data from experiments performed *in vitro* in BC and HCC cancer cell lines as well as human BC tissue samples. A diagrammatic representation of the interactions analyzed in this review is shown in *Figure 1*.
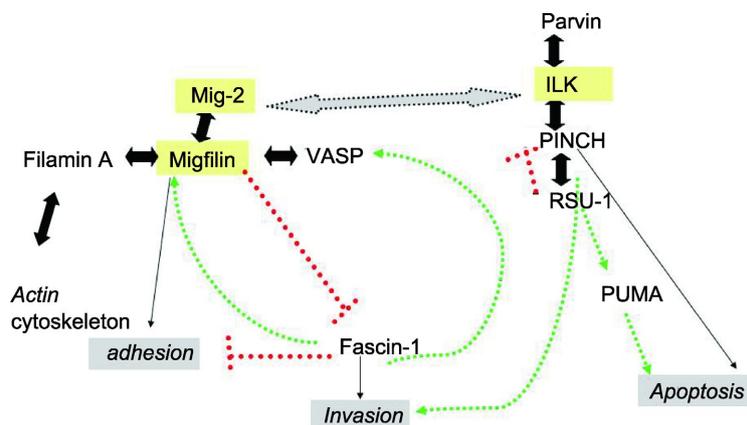
Figure 1 Diagrammatic representation of the protein-protein interactions studied in the present review and their involvement in different aspects of cancer cell metastasis

The role of migfilin in cancer cell metastasis

Several novel molecules at cell-ECM adhesion sites have been implicated in cancer cell progression and have been recently shown to be deregulated in cancer cell metastasis.

Migfilin, (also known as filamin-binding LIM protein 1 or FBLP-1), is a novel LIM domain—containing protein present both at cell-ECM[7] and cell-cell adhesions[8]. It interacts with the cell-ECM adhesion protein mitogen-inducible gene 2 (Mig-2)[7] and it also binds to vasodilator-stimulated phosphoprotein (VASP)[9] which is known to regulate actin polymerization in lamellipodia[10-12], the cellular protrusions responsible for migration and invasion of the cell. Migfilin has been associated with various types of cancer although its role has not been extensively studied. Cytoplasmic migfilin was strongly associated with higher tumor grade in leiomyosarcomas[13] and inversely correlated with clinical metastasis in esophageal cancer cells[14]. Moreover, high migfilin expression was significantly correlated with tumor grade in glioma and poor prognosis[15]. Finally, work from our group has shown that migfilin was also significantly reduced in human BC samples compared to normal adjacent tissue[16], indicating an involvement of the protein in cancer progression. Along the same line, migfilin was also examined in advanced-stage serous ovarian carcinoma[17] and was found to have significantly lower expression in primary carcinomas and solid metastases compared to effusions[17]. However, although as mentioned above, recent studies have shown that migfilin's expression is negatively correlated with clinical metastasis in esophageal cancer[14], we found no statistically correlation between migfilin's expression and metastatic

status or disease stage in human BC samples[16]. As metastasis is a fundamental biological behavior of HCC and the main cause of treatment failure[2], we also tested the *in vitro* role of migfilin in two liver cell lines that differ in terms of their metastatic potential; the non-invasive hepatoma cell line PLC/ PRF/5 (Alexander cells herein) and the highly invasive HCC HepG2 cell line[18]. Using an siRNA-mediated silencing approach to knock-down the *migfilin* gene from both cell lines, we studied the effect of gene silencing on basic signaling pathways and functional cellular properties related to the metastatic potential of the cells. We found that migfilin was elevated in the more invasive HepG2 cells compared to the less invasive Alexander cells both at the mRNA and protein level, indicating a possible contribution of the protein to the aggressive phenotype of HepG2 cells[18]. Moreover, gene silencing of *migfilin* led to upregulation of proteins involved in actin reorganization such as the two main forms of phosphorylated VASP (Ser157 and Ser239) in HepG2 cells, and Fascin-1 and Rho kinase (ROCK-1) in both cell lines. Increased expression of these molecules led to increased actin polymerization and stabilization, which thus resulted in less available monomeric actin for cell migration or invasion ultimately contributing to reduced cell migration[19,20]. Indeed, migfilin depletion led to reduced cell invasion, indicating that migfilin promotes the metastatic phenotype of HepG2 cells[18]. In fact, this is in accordance with previous studies showing migfilin to be crucial for cell migration in a variety of cell types (HeLa, HT-1080, and MDA-MB-231 cells), where it was shown that its depletion impairs cell migration[9]. As migfilin has been previously shown to link the cell-matrix adhesions to the actin cytoskeleton[7], the migratory defect induced by the loss of migfilin is probably caused, at least in part, by the im-

paired connection between cell-ECM adhesions and the actin cytoskeleton. A diagrammatic representation of the interactions mentioned above is shown in *Figure 1*.

Notably, the fact that elimination of migfilin severely impaired HepG2 cell invasion, while at the same time increasing cell proliferation, both through elevation of ERK1/2 and phosphor-β-catenin[18], indicates that migfilin has a dual function in HCC cells activating different signaling pathways perhaps through interaction with different binding partners. This could provide an explanation as to why its role in cancer is not strictly defined but greatly depends upon the type of cancer[14,16,17,21]. Nevertheless, evidence suggests that migfilin is worth being assessed as a therapeutic target for cancer cell metastasis for a number of cancer types.

Mitogen-inducible gene-2 (Mig-2) in cancer cell metastasis

Mitogen inducible gene-2 (Mig-2) is a cell-ECM adhesion protein localized at cell-ECM adhesion sites[22], and through its binding partner, migfilin[23], interacts with filamin A, thus connecting cell-ECM adhesions to the actin cytoskeleton (*Figure 1*)[7]. Recent studies have implicated Mig-2 along with migfilin in a variety of human cancers. Mig-2 expression was found increased in leiomyomas compared with normal myometrium while it was decreased in leiomyosarcomas[24]. It was also upregulated in gastric cancer and its expression had a significant positive correlation with metastasis and poor survival[25]. Mig-2 was also shown to be highly expressed in 90% of malignant mesothelioma tumors[26] as well as in almost 100% of human bladder cancers[27] and in the majority of chondrosarcomas[28]. In fact, it has been postulated that Mig-2 could function as a promising marker of tumor progression[25,28]. Finally, Mig-2 deregulation[29] could contribute to BC malignancy and progression. Previous work from our research group[16] has actually shown that Mig-2 expression was significantly reduced in human BC tissues compared to normal adjacent tissue indicating that BC has lost Mig-2 expression and thereby has less invasion inhibitory mechanisms in action, as Mig-2 has been implicated in inhibition of cell invasion[30].

Ras suppressor-1 (RSU-1) in metastasis

Mig-2 has been also shown in *C. elegans* to interact with integrin-linked kinase (ILK)[31] thus being connected to particularly interesting new cysteine-histidine rich pro-

tein-1 (PINCH-1) and parvin, forming a stable ternary protein complex at cell-ECM adhesion sites[32]. Although many studies have been performed on the role of ILK in cancer, even proposing that it is a potential anti-cancer therapeutic target[33,34], little is known with regard to the ILK-interacting partners. PINCH-1 in particular, functions as an adaptor protein at the cell-ECM adhesion sites playing an important role in promoting cell survival and apoptosis resistance[35], while also modulating cell shape and spreading[36]. Interestingly, PINCH-1 interacts with Ras suppressor-1 (RSU-1) at the cell-ECM adhesion sites[37,38], increasing the sites' complexity. RSU-1 was originally identified as a suppressor of Ras-dependent oncogenic transformation[39]. Although, its connection to cancer is obvious due to its linkage with Ras oncogene, little is known regarding its involvement in the disease. It has been shown that RSU-1 is involved in the inhibition of anchorage independent growth of BC cells[40,41]. Moreover, in a recent study by our group it was shown that RSU-1 is upregulated in HepG2 highly invasive HCC cells compared to the more benign, non-invasive Alexander hepatoma cells, which indicates a possible involvement of RSU-1 in HCC pathogenesis[42]. Moreover, siRNA-mediated gene silencing of RSU-1 expression in both HCC cell lines resulted in increased proliferation, reduced cell adhesion and cell invasion in the aggressive HepG2 cells[42], suggesting that RSU-1 enhances adhesion and invasion in the aggressive HepG2 cells. Interestingly, in another recent study by our group, RSU-1 was investigated in BC cell lines that differ in terms of their metastatic potential (namely, non-invasive MCF-7 cells and highly invasive MDA-MB-231 cells) as well as in a set of thirty two[32] human BC samples from patients with or without lymph node metastasis[43]. In this study, the findings from the experiments performed in the cell lines were further supported by the findings in the human samples validating the hypothesis that RSU-1 plays a vital role in BC metastasis. More specifically, RSU-1 was found to be upregulated in the more aggressive MDA-MB-231 cells when compared to the less transformed MCF-7 cells, connecting RSU-1 with a more aggressive BC phenotype. Furthermore, it was shown that depletion of RSU-1 leads to upregulation of its binding partner PINCH-1, indicating that RSU-1 likely acts as a negative regulator of pro-survival ECM-adhesion protein PINCH-1. This was also in accordance with the fact that silencing of RSU-1 enhanced cell proliferation, which is not surprising, as RSU-1 was originally characterized as a suppressor of Ras-dependent oncogenic transformation[39]. Interestingly, RSU-1 depletion significantly reduced the population of apop-

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totic cells indicating that RSU-1 actually promotes apoptosis. Consistent with this change, RSU-1 depletion, from both cell lines, also led to downregulation of the mRNA expression of the pro-apoptotic gene p53 upregulated modulator of apoptosis (PUMA). More importantly, the findings in the two BC cell lines were further validated in 32 human BC samples from patients with invasive BC with or without lymph node metastasis and each sample was accompanied by and compared to its corresponding normal adjacent tissue, eliminating any external factors and variations that might interfere with the results. Interestingly, the data showed that RSU-1 mRNA expression was significantly increased in BC tissues when compared to normal adjacent tissue. Moreover, RSU-1 expression was significantly reduced in non-metastatic samples and significantly elevated in metastatic samples, clearly stating a different role of RSU-1 depending on the aggressiveness of the tumor, that could potentially lead to the use of RSU-1 as a novel biomarker of metastasis[43]. In accordance with the cell line data that suggested RSU-1 to be a negative regulator of PINCH-1, PINCH-1 expression showed dramatic reduction in BC samples both at the mRNA and protein level with statistically significant reduction in metastatic samples, in which RSU-1 was found to be elevated. Moreover, the assessment of the pro-apoptotic PUMA expression also confirmed findings in the cell lines showing an increase of PUMA expression in BC and metastatic samples in particular and a positive statistically significant correlation with RSU-1 expression in the same samples[43].

Therefore, RSU-1 should definitely be evaluated further as a potential metastasis biomarker in BC tissue samples. Moreover, new therapeutic approaches that reinforce apoptosis through PUMA should also be assessed in comparison to current available therapies in patients with BC metastasis.

Cell adhesion and endothelial cell growth in metastasis

Moreover, deregulation of focal adhesion proteins combined with an imbalance between pro- and anti-angiogenic signaling in cells[44] also affects tumor vasculature, which is another critical factor in tumor growth and metastasis. More specifically, the endothelial cell lining in tumor vessels consists of less junction proteins when compared to the endothelial cell lining in normal vessels leaving large numbers of fenestrae and intercellular openings, being accompanied, at the same time by a disorganized ECM and basement membrane[45].

As a result, tumor vessels become "leaky" facilitating the spreading of circulating tumor cells throughout the whole body. To this regard, pro-angiogenic factors (e.g. vascular endothelial growth factor, VEGF, platelet derived growth factor, PDGF) and their receptors are upregulated in most tumors while focal adhesion proteins are mainly downregulated resulting in loose cell-ECM connections and the formation of immature vessels with structural abnormalities. Interestingly, there has been no connection between either of the molecules under review (namely, Mig-2, migfilin, or RSU-1) and tumor angiogenesis, leaving the field open for future investigations.

Conclusion

In the current review, we have summarized the recent data on novel and interesting proteins found at the cell-ECM adhesion sites of cells, namely migfilin, Mig-2 and RSU-1, and their involvement in cancer cell metastasis emphasizing on results from BC and HCC studies. We conclude that these cell-ECM adhesion proteins tend to function as adaptor proteins forming multiple protein-protein interactions at the cell-ECM adhesion sites, thus conferring different effects on different cancer cell types. However, in most cases, and as shown in *Figure 1*, they promote cell adhesion, cell invasion and apoptosis, all of which are important aspects of cancer cell metastasis. Therefore, more research is needed involving more human samples in order to evaluate their use as potential biomarkers of metastasis and perhaps, even potential targets for anticancer therapy.

Conflict of interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

References

1. Weigelt B, Peterse JL, van't Veer LJ. Breast cancer metastasis: markers and models. Nat Rev Cancer 2005; 5(8): 591–602.
2. Li Y, Tang ZY, Hou JX. Hepatocellular carcinoma: insight from animal models. Nat Rev Gastroenterol Hepatol 2011; 9(1): 32–43.
3. Kakonen SM, Mundy GR. Mechanisms of osteolytic bone metastases in breast carcinoma. Cancer 2003; 97(Suppl 3): 834–839.
4. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002; 110(6): 673–687.
5. Canel M, Serrels A, Frame MC, et al. E-cadherin-integrin

crosstalk in cancer invasion and metastasis. J Cell Sci 2013; 126(Pt 2): 393–401.

6. Ungefroren H, Sebens S, Seidl D, et al. Interaction of tumor cells with the microenvironment. Cell Commun Signal 2011; 9: 18.

7. Tu Y, Wu S, Shi X, et al. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. Cell 2003; 113(1): 37–47.

8. Gkretsi V, Zhang Y, Tu Y, et al. Physical and functional association of migfilin with cell-cell adhesions. J Cell Sci 2005; 118(Pt 4): 697–710.

9. Zhang Y, Tu Y, Gkretsi V, et al. Migfilin interacts with vasodilator-stimulated phosphoprotein (VASP) and regulates VASP localization to cell-matrix adhesions and migration. J Biol Chem 2006; 281(18): 12397–12407.

10. Castellano F, Le Clainche C, Patin D, et al. A WASp-VASP complex regulates actin polymerization at the plasma membrane. EMBO J 2001; 20(20): 5603–5614.

11. Sechi AS, Wehland J. ENA/VASP proteins: multifunctional regulators of actin cytoskeleton dynamics. Front Biosci 2004; 9: 1294–1310.

12. Kwiatkowski AV, Gertler FB, Loureiro JJ. Function and regulation of Ena/VASP proteins. Trends Cell Biol 2003; 13(7): 386–392.

13. Papachristou DJ, Gkretsi V, Tu Y, et al. Increased cytoplasmic level of migfilin is associated with higher grades of human leiomyosarcoma. Histopathology 2007; 51(4): 499–508.

14. He H, Ding F, Li Y, et al. Migfilin regulates esophageal cancer cell motility through promoting GSK-3 β-mediated degradation of β-catenin. Mol Cancer Res 2012; 10(3): 273–281.

15. Ou Y, Ma L, Dong L, et al. Migfilin protein promotes migration and invasion in human glioma through epidermal growth factor receptor-mediated phospholipase C-γ and STAT3 protein signaling pathways. J Biol Chem 2012; 287(39): 32394–32405.

16. Gkretsi V, Papanikolaou V, Zacharia LC, et al. Mitogen-inducible Gene-2 (MIG2) and migfilin expression is reduced in samples of human breast cancer. Anticancer Res 2013; 33(5): 1977–1981.

17. Davidson B, Holth A, Nguyen MT, et al. Migfilin, α-parvin and β-parvin are differentially expressed in ovarian serous carcinoma effusions, primary tumors and solid metastases. Gynecol Oncol 2013; 128(2): 364–370.

18. Gkretsi V, Bogdanos DP. Experimental evidence of Migfilin as a new therapeutic target of hepatocellular carcinoma metastasis. Exp Cell Res 2015; 334(2): 219–227.

19. Maekawa M, Ishizaki T, Boku S, et al. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science 1999; 285(5429): 895–898.

20. Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol 2003; 4(6): 446–456.

21. He H, Ding F, Li S, et al. Expression of migfilin is increased in esophageal cancer and represses the Akt-β-catenin activation. Am J Cancer Res 2014; 4(3): 270–278.

22. Mackinnon AC, Qadota H, Norman KR, et al. C. elegans PAT-4/ILK functions as an adaptor protein within integrin adhesion complexes. Curr Biol 2002; 12(10): 787–797.

23. Sepulveda JL, Gkretsi V, Wu C. Assembly and signaling of adhesion complexes. Curr Top Dev Biol 2005; 68: 183–225.

24. Kato K, Shiozawa T, Mitsushita J, et al. Expression of the mitogen-inducible gene-2 (mig-2) is elevated in human uterine leiomyomas but not in leiomyosarcomas. Hum Pathol 2004; 35(1): 55–60.

25. Shen Z, Ye Y, Dong L, et al. Kindlin-2: a novel adhesion protein related to tumor invasion, lymph node metastasis, and patient outcome in gastric cancer. Am J Surg 2012; 203(2): 222–229.

26. An Z, Dobra K, Lock JG, et al. Kindlin-2 is expressed in malignant mesothelioma and is required for tumor cell adhesion and migration. Int J Cancer 2010; 127(9): 1999–2008.

27. Talaat S, Somji S, Toni C, et al. Kindlin-2 expression in arsenite- and cadmium-transformed bladder cancer cell lines and in archival specimens of human bladder cancer. Urology 2011; 77(6): 1507.e1–e7.

28. Papachristou DJ, Gkretsi V, Rao UN, et al. Expression of integrin-linked kinase and its binding partners in chondrosarcoma: association with prognostic significance. Eur J Cancer 2008; 44(16): 2518–2525.

29. Gozgit JM, Pentecost BT, Marconi SA, et al. Use of an aggressive MCF-7 cell line variant, TMX2-28, to study cell invasion in breast cancer. Mol Cancer Res 2006; 4(12): 905–913.

30. Shi X, Wu C. A suppressive role of mitogen inducible gene-2 in mesenchymal cancer cell invasion. Mol Cancer Res 2008; 6(5): 715–724.

31. Xu X, Rongali SC, Miles JP, et al. pat-4/ILK and unc-112/Mig-2 are required for gonad function in *Caenorhabditis elegans*. Exp Cell Res 2006; 312(9): 1475–1483.

32. Wu C. The PINCH-ILK-parvin complexes: assembly, functions and regulation. Biochim Biophys Acta 2004; 1692(2–3): 55–62.

33. Legate KR, Montanez E, Kudlacek O, et al. ILK, PINCH and parvin: the tIPP of integrin signalling. Nat Rev Mol Cell Biol 2006; 7(1):20–31.

34. Wickström SA, Lange A, Montanez E, et al. The ILK/PINCH/parvin complex: the kinase is dead, long live the pseudokinase. EMBO J 2010; 29(2): 281–291.

35. Chen K, Tu Y, Zhang Y, et al. PINCH-1 regulates the ERK-Bim pathway and contributes to apoptosis resistance in cancer cells. J Biol Chem 2008; 283(5): 2508–2517.

36. Xu Z, Fukuda T, Li Y, et al. Molecular dissection of PINCH-1 reveals a mechanism of coupling and uncoupling of cell shape modulation and survival. J Biol Chem

2005; 280(30): 27631–27637.

37. Dougherty GW, Chopp T, Qi SM, et al. The Ras suppressor Rsu-1 binds to the LIM 5 domain of the adaptor protein PINCH1 and participates in adhesion-related functions. Exp Cell Res 2005; 306(1): 168–179.

38. Kadrmas JL, Smith MA, Clark KA, et al. The integrin effector PINCH regulates JNK activity and epithelial migration in concert with Ras suppressor 1. J Cell Biol 2004; 167(6): 1019–1024.

39. Cutler ML, Bassin RH, Zanoni L, et al. Isolation of rsp-1, a novel cDNA capable of suppressing v-Ras transformation. Mol Cell Biol 1992; 12(9): 3750–3756.

40. Dougherty GW, Jose C, Gimona M, et al. The Rsu-1-PINCH1-ILK complex is regulated by Ras activation in tumor cells. Eur J Cell Biol 2008; 87(8–9): 721–734.

41. Vasaturo F, Dougherty GW, Cutler ML. Ectopic expression of Rsu-1 results in elevation of p21CIP and inhibits anchorage-independent growth of MCF7 breast cancer cells. Breast Cancer Res Treat 2000; 61(1): 69–78.

42. Gkretsi V, Bogdanos DP. Elimination of Ras Suppressor-1 from hepatocellular carcinoma cells hinders their *in vitro* metastatic properties. Anticancer Res 2015; 35(3): 1509–1512.

43. Giotopoulou N, Valiakou V, Papanikolaou V, et al. Ras suppressor-1 promotes apoptosis in breast cancer cells by inhibiting PINCH-1 and activating p53-upregulated-modulator of apoptosis (PUMA); verification from metastatic breast cancer human samples. Clin Exp Metastasis 2015; 32(3): 255–265.

44. Wang Y, Simpson JA, Wluka AE, et al. Relationship between body adiposity measures and risk of primary knee and hip replacement for osteoarthritis: a prospective cohort study. Arthritis Res Ther 2009; 11(2): R31.

45. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011; 147(2): 275–292.